VOLUME - 10, ISSUE - 05, MAY- 2021 • PRINT ISSN No. 2277 - 8160 • DOI : 10.36106/gjra

Original Research Paper

Dental Science

THE ROLE OF INTERMEDIATE IRRIGANTS IN THE PREVENTION OF THE FORMATION OF ORANGE-BROWN PRECIPITATE, FORMED DUE TO INTERACTION BETWEEN SODIUM HYPOCHLORITE AND CHLORHEXIDINE: A SPECTROPHOTOMETRIC ANALYSIS.

Dr Annapoorna*	Professor, Department of Conservative Dentistry and Endodontics, Rajarajeswari Dental college and Hospital, Bengaluru, India. *Corresponding Author
Dr Manjunatha M	Professor, Department of Conservative Dentistry and Endodontics, Syamala Reddy Dental college and Hospital, Bengaluru, India.
Dr Shubhashini N	Professor, Department of Conservative Dentistry and Endodontics, Rajarajeswari Dental college and Hospital, Bengaluru, India.
Dr Blesy V	Post graduate, Department of Conservative Dentistry and Endodontics, Rajarajeswari Dental college and Hospital, Bengaluru, India.
Dr Keerthana S	Post Graduate, Department of Conservative Dentistry and Endodontics, Rajarajeswari Dental college and Hospital, Bengaluru, India.
Dr Prerana K	Post Graduate, Department of Conservative Dentistry and Endodontics, Rajarajeswari Dental college and Hospital, Bengaluru, India.

ABSTRACT

This study aimed to assess the efficiency of intermediate irrigants in the prevention of formation of precipitate, formed due to the interaction of sodium hypochlorite and chlorhexidine when used as root canal irrigants.The formation of orange brown precipitate, due to the reaction of 3% sodium hypochlorite and 2% chlorhexidine was assessed using spectrophotometric analysis. Three intermediate irrigants were then assessed for their capabilities to prevent the formation of the precipitate. Group A- 6.25% sodium metabisulphite, Group B- 10% sodium ascorbate and Group C- saline were used at different volumes and the values were recorded by measuring their optical density. An invitro analysis was further carried out on single rooted teeth, to verify the efficacy of intermediate irrigants. The teeth which were subjected to the tests were sectioned and observed under stereomicroscope for the presence of remnants of orange brown precipitate in the root canals.

Results: The spectrophotometric analysis showed peak value at 450nm and the precipitate formation was found at 60μl of 2% chlorhexidine and 1 ml of sodium hypochlorite. There was statistical difference in the optical density values between the groups, with saline having highest optical density values followed by 6.25% sodium metabisulphite and 10% sodium ascorbate group. Wherein 10% sodium ascorbate solution prevented the formation of orange brown precipitate than other two group. It was concluded that 10% sodium ascorbate played a vital role in the reduction of formation of parachloro-aniline, when used as an intermediate irrigant. 6.25% Sodium metabisulphite also prevented the precipitate formation, to a lesser extent than sodium ascorbate. However, saline did not eliminate the formation of the precipitate.

KEYWORDS : parachloro aniline. Intermediate irrigants, orange-brown precipitate

INTRODUCTION

Complete elimination of microorganisms that cause periapical inflammation, is one of the primary objective of root canal therapy. The anatomic complexities of the root canal systems prevent mechanical instrumentation and removal of all infected tissues in the isthmus. This indicates the importance of irrigants alongside mechanical instrumentation. Root canal irrigants come in contact with each other in the canal systems which increases the formation of byproducts. The byproducts formed can form a barrier between the dentin and root canal sealer preventing close adaptation of obturating materials. This can increase microleakage and the byproducts could be toxic to periapical tissues[1,2].

The association between sodium hypochlorite and chlorhexidine is seen to produce orange- brown precipitate. The chemical composition of this precipitate is thought to be parachloro-aniline by some authors or to be parachlorphenyl urea and parachlorophenylguanidyl-1.6-diguanidyl-hexane (PCGH) by others[3]. The by-products are known to be carcinogenic and mutagenic. To avoid the formation of these precipitates various intermediate irrigants have been recommended. 10% sodium ascorbate has been proven as an effective root canal irrigant[4] with substantial antimicrobial activity. 6.25% sodium metabisulphite has been proven to reduce precipitate formation in the root canal hence is used in this study as a positive control.[5]

The aim of this study is to verify by spectrophotometric analysis, the formation of parachloroaniline precipitate and compare the efficacy of intermediate irrigants in prevention of formation of the precipitate. The various intermediate irrigants used in this study are saline, 6.25% sodium metabisulphite and 10% sodium ascorbate.

MATERIALS AND METHODS

The study was conducted at Dextrose Technologies Pvt Ltd., Bangalore, Karnataka, India. All the chemicals, test samples were procured from Himedia (Mumbai). 3% Sodium hypochlorite, 2% Chlorhexidine, 6.25% Sodium Metabisulphite, Saline, 10% Sodium Ascorbate was prepared just before the analysis.

λ_{max} estimation of Parachloroaniline:

Inorder to estimate the λ_{max} of Parachloroaniline, 1 ml of 3% sodium hypochlorite and increasing volume of chlorhexidine solution (10-100μl) were mixed to find the concentration of chlorohexidine at which orange-brown precipitate, was formed. Following which that particular concentration was obtained and analysed spectrophotometrically at a wavelength range between 350-550 nm and λ_{max} was calculated.

According to three different intermediate irrigating solutions, 6.25% sodium metabisulphite,10% sodium ascorbate, saline were grouped as A, B, C respectively. Two trials for each group of irrigating solution (test samples) was carried out spectrophotometrically, using centrifuged test sample. The test mixture was centrifuged at 5000 rpm for 20 minutes.

Group A: 100μl of 3% Sodium hypochlorite and 60μl of 2% Chlorhexidine was taken as control and was tested against centrifuged test solution containing 100μl of 3% Sodium hypochlorite, 60μl of 2% Chlorhexidine and different volumes of 6.25% Sodium Metabisulphite (10-100μl) by measuring the Optical Density

Group B: 100μl of 3% Sodium hypochlorite and 60μl of 2% Chlorhexidine was taken as control and was tested against centrifuged test solution containing 100μl of 3% Sodium hypochlorite, 60μl of 2% Chlorhexidine and different volumes of 10% Sodium Ascorbate(10-100μl) by measuring the Optical Density

Group C: 100μl of 3% Sodium hypochlorite and 60μl of 2% Chlorhexidine was taken as control and was tested against centrifuged test solution containing 100μl of 3% Sodium hypochlorite, 60μl of 2% Chlorhexidine and different volumes of saline (10-100μl) by measuring the Optical Density.

In vitro analysis:

In vitro analysis was carried out using single rooted teeth. After washing with distilled water and ultrasonic scaling, the specimen were immersed in 0.5% Chloramine T solution until use. The teeth were decoronated to obtain a standardized root length. Canal patency was evaluated using #10 K file and teeth with canal obstructions discarded. The actual length of each tooth was determined with #10 K file, which was introduced into the canal until its tip emerged through the major apical foramen. The working length was established by subtracting 1 mm from the actual length. The apices of the specimen were then sealed with wax to prevent extrusion of irrigating solutions. All the canals were prepared by using NiTi rotary files until F4 size as per the manufacturer's instructions. And the canals were irrigated with 3%NaOCl between each instrumentation sequence using a 30G side vented needle, introduced 2 mm short of the apex. After complete mechanical instrumentation all the samples were thoroughly flushed with 2.5 ml of 3% NaOCl using 30G side vented needle. The samples were randomly allocated to three groups of 15 samples each based on the use of 2.5 ml of intermediate irrigating solution for 60 seconds as specified below.

Group A-6.25% Sodium Metabisulphite, Group B –10% sodium ascorbate, Group C-Saline(control). After which, all the group samples were irrigated with 2 ml of 2% CHX gluconate solution as final wash. The root canals were dried using paper points of corresponding apical preparation size.

Two longitudinal grooves were made along the buccal and lingual surfaces of the roots with water-cooled diamond disc. The roots were then sectioned with a chisel and was observed under stereomicroscope.

Statistical analysis: Statistics was carried out using Statistical Package for Social Sciences [SPSS] for Windows, Version 22.0. Released 2013. Armonk, NY: IBM Corp. Descriptive Statistics was taken to correlate expression of the OD values in terms of Mean and SD. And Inferential Statistics One-way ANOVA followed by Tukey's Post hoc Analysis was used to compare the mean Optical Density values between 3 groups. The level of significance [P-Value] was set at $P<0.05$.

RESULTS:

λ_{max} estimation of Parachloroaniline

Parachloroaniline formation (orange-brown precipitation) was found at a concentration of 60μl of chlorhexidine and 1ml of Sodium hypochlorite (Figure 1). The spectrophotometric analysis showed peak value at 450nm that is the intensity of orange brown precipitate formation. (Graph 1)

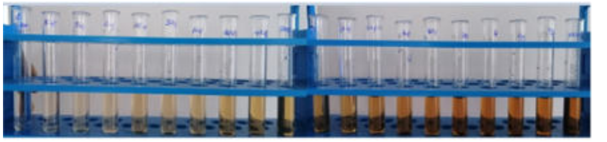
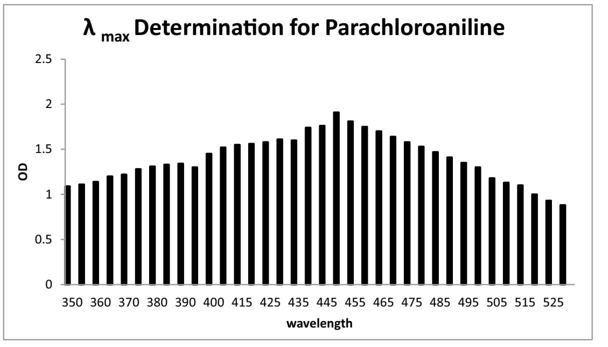


Figure 1 – Determination of parachloroaniline precipitate by increasing concentration of chlorohexidine from 10μl -100μl with 100 μl of 3% sodium hypochlite.



Graph 1- Wavelength scan for determination of parachloroaniline peak using spectrophotometric method (350-550nm)

Testing of irrigating solutions

Group A: 6.25% Sodium Metabisulfite

Para-chloroaniline formation was decreasing upon increasing the volume of sodium metabisulphite and it was found that at a volume of 60μl of sodium metabisulphite decreased the orange- brown precipitate formation. (Fig 2)

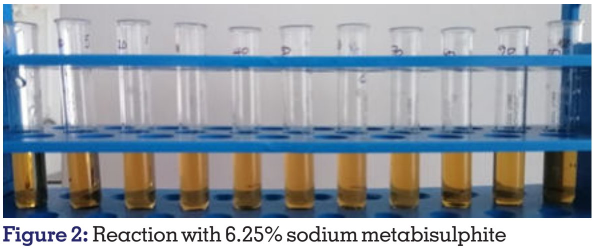


Figure 2: Reaction with 6.25% sodium metabisulphite

Group B: 10% sodium ascorbate: Para-chloroaniline formation was decreasing upon increasing the volume of sodium ascorbate and it was found that at a volume of 40μl of sodium ascorbate decreased the orange- brown precipitate formation.(Fig 3)

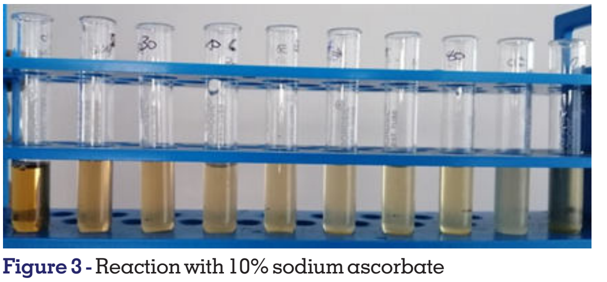


Figure 3 - Reaction with 10% sodium ascorbate

Group C: Saline

Increasing the amount of saline did not prevent or decrease the formation of the precipitate. (Fig 4)

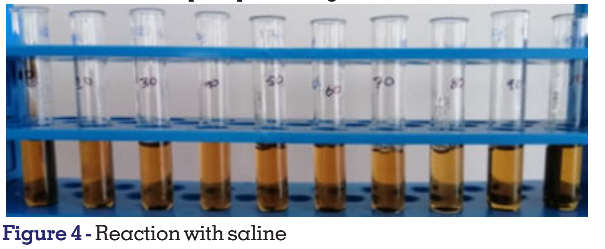


Figure 4 - Reaction with saline

The test demonstrates the mean Optical Density values measured for each study group. The mean Optical Density values for 6.25% Sodium Meta-bisulphite group was 0.6058 ± 0.0928, for 10% Sodium Ascorbate group, was 0.5601 ± 0.0426 and for Saline group, it was 0.6871 ± 0.0419. This difference in the mean Optical Density values between the groups was statistically significant at P=0.001. [Refer Table no. 1& Graph 2]

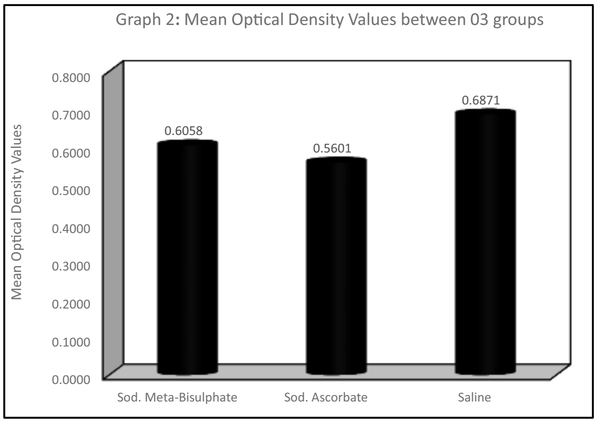


Table no. 1: Comparison of mean Optical Density values between the groups using One-way ANOVA Test

Groups	N	Mean	SD	Min	Max	P-Value
Sod. Meta Bisulphite	10	0.6058	0.0928	0.504	0.760	0.001*
Sod. Ascorbate	10	0.5601	0.0426	0.484	0.611	
Saline	10	0.6871	0.0419	0.642	0.761	

* - Statistically Significant

Multiple comparison of mean differences between different groups revealed that Saline group showed significantly higher mean Optical Density values as compared to 6.25% Sodium Meta-bisulphite group and 10% Sodium Ascorbate group at P=0.02 & P=0.001 respectively. This was followed by 6.25% Sodium Meta-bisulphite group showing relatively higher mean Optical Density values as compared to 10% Sodium Ascorbate group. However, no significant differences were observed between these 2 groups [P=0.26]. This infers that the mean Optical Density values is highest in Saline group followed by 6.25% Sodium Meta-bisulphite group and least in 10% Sodium Ascorbate group. [Refer table no. 2 & Graph 3]

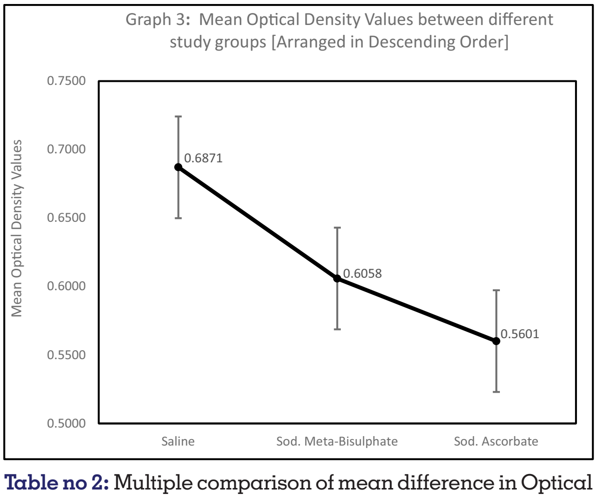
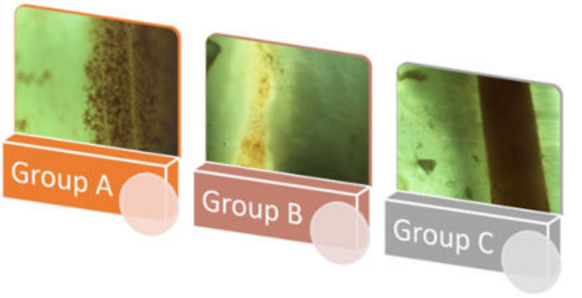


Table no 2: Multiple comparison of mean difference in Optical Density values between 03 groups using Tukey's Post hoc Analysis

Groups(I)	Groups(J)	Mean Diff. (I-J)	95% CI for the Diff.		P-Value
			Lower	Upper	
Sod. Meta Bisulphite	Sod. Ascorbate	0.0458	-0.0249	0.1164	0.26
	Saline	-0.0813	-0.1519	-0.0106	0.02*
Sod. Ascorbate	Saline	-0.1270	-0.1977	-0.0563	0.001*

* - Statistically Significant

Stereomicroscopic image of tooth section (Figure 5) A) Sodium meta bisulphite B) Sodium ascorbate C) Saline are seen.



DISCUSSION:

Bacteria and their byproducts are the main causative factors to initiate and cause periapical inflammation. Hence one of the main goal is to reduce the microorganisms in the canal by using combination of root canal irrigants. An irrigating solution is not completely flushed out from the root canal before next irrigating solution is placed. Hence they come into contact with each other in the root canal[6,7]. This will lead to interaction of the irrigants, formation of precipitates that can lead to tooth discoloration and interfere with sealing of root canal fillings. Use of sodium hypochlorite and chlorhexidine as root canal irrigants was seen to form orange brown precipitate. These by-products are considered to be mutagenic and carcinogenic[8,9].

Chlorhexidine when placed in an aqueous solution, hydrolyses due to substitution of guanidine group. When chlorhexidine and sodium hypochlorite is mixed, the chlorhexidine molecules become hydrolysed into smaller fragments. The first bonds to be broken are between carbon and nitrogen because of low bond dissociation energy[10-12]. The precipitate formation is also explained by acid-base reaction, when sodium hypochlorite and chlorhexidine is mixed. Chlohexidine is a dicatonic acid, with a pH of 5.5-6. These have ability to donate protons. Sodium hypochlorite is alkaline and can accept protons from chlorhexidine. This proton exchange results in formation of neutral insoluble precipitate[11].

Hence, to avoid formation of precipitates, intermediate intracanal flush is recommended. In this study the intermediate irrigants used were sodium metabisulphite and sodium ascorbate.

Sodium metabisulphite is chemical reagent that is used as disinfectant, antioxidant and preservative. The performance of sodium metabisulphite in minimisng the precipitate formation is related to the neutralizing sodium hypochlorite by removing free chlorine ions. Weak acid salt, hypochlorous acid, is formed due to oxidation reaction converting sulfite group to sulfate group[5,13,14].

Sodium ascorbate is a salt form of ascorbic acid. It is a potent antioxidant that is capable of quenching reactive free radicals in the biological systems. Sodium ascorbate neutralizes adverse effects of oxidizing solutions via redox reactions. It is also proven that sodium ascorbate neutralizes chlorine.[16] 10% sodium ascorbate has been used on a large scale for neutralizing chlorine in water systems and has been proven safe.[17]

Within the limits of this study, the orange brown precipitate formed due to interaction between sodium hypochlorite and chlorhexidine can be eliminated with the use of intermediate irrigant flushes. In this study, it was found that 10% sodium ascorbate prevented formation of byproducts than sodium metabisulphite. Since sodium ascorbate is a salt of vitamin C, no undesirable effects is expected and can be safely used as an intermediate irrigating solution. It can be used as an alternative to other intermediate irrigating solutions currently used and gives insight about the analysis and novel ways of prevention of by-products during root canal treatment.

CONCLUSION:

In this study, it can be concluded that 10% sodium ascorbate played a vital role in the reduction of formation of parachloro-aniline, when used as an intermediate irrigant. 6.25% Sodium metabisulphite also prevented the precipitate formation, to a lesser extent than sodium ascorbate. However, saline did not eliminate the formation of the precipitate.

Acknowledgements:

We thank everyone who supported this study, especially Dr. Sumangala Bhat and Miss Aiswarya from Dextrose Technologies pvt. Ltd. for allowing to carrying out the study at their company.

REFERENCES:

1. Basrani B R , Manek S, Sodhi R N, et al. interaction between sodium hypochlorite and chlorheidine gluconate. J Endod 2007;33:966-9.
2. Rasimick BJ, Nekich M, Hladek MM. Interaction between chlorhexidine gluconate and EDTA. J Endod 2008;34:1521-3.
3. Nowicki JB, Sem DS. An invitro spectroscopic analysis to determine the chemical composition of the precipitate formed by mixing sodium hypochlorite and chlorhexidine. J Endod 2011;37:983-8.
4. Charles H Weston, Shuichi Ito, Bakul Wadgaonkar, David H Pashley. Effects of time and concentration of sodium ascorbate on reversal of sodium hypochlorite induced reduction in bond strengths. J Endod 2007: 33(7), 879-81.
5. Bui T, Baumgartner J, Mitchell J. evaluation of the interaction between sodium hypochlorite and chlorhexidine gluconate and its effect on root dentin. J Endod2008;34:181-5.
6. Naveen Chhabra, Satish G , Kiran PS, Krupa D, Kritika G. Efficacy of various solutions in preventing orange-brown precipitate formed during alternate use of sodium hypochlorite and chlorhexidine : An invitro study. J Cons Dentistry 2018;21(4):428-32.
7. Baumgartner JC, Ibay AC. The chemical reactions of irrigants used for root canal debridment. J Endod 1987;13:47-51.
8. Burkhardt-Holm P, Oulmi Y, Schroeder A, Storch V, Braunbeck T. toxicity of 4-chloroaniline in early life stages of zebrafish (Danio rerio): II. Cytopathology and regeneration of liver and gills after prolonged exposure to waterborne 4-chloroaniline. Arch Environ Contam Toxicol 1999, 37:85-102.
9. Yildirim T, Er K, Tasdemir T, Tahan E, Buruk K, Serper A. effect of smear layer and root end cavity thickness on apical sealing ability of MTA as a root end filling material: a bacterial leakage study. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2010;109:e67-72.
10. Heard DD, Ashworth RW. The colloidal properties of chlorhexidine and its integration with some macromolecules. J Pharm Pharmac 1968;20:505-12.
11. Goodall R, Goldman J, Woods J. Stability of chlorhexidine solutions. Pharm J 1968;13:33-4.
12. Fessenden R, Fessenden J. Organic chemistry. Boston: Willard Grant Press; 1997.
13. Sodhi R, Grad H. Examination by x-ray photoelectron spectroscopy of the adsorption of of chlorhexidine on hydroxyapatite. J Dent Res 1992;71:493-7.
14. Noorafshan A, Asadi-Golshan R, Monjezi S, Karbalay-Doust S. sodium metabisulphite, a preservative agent, decreases the heart capillary volume and length, and curcumin, the main component of curcuma longa, cannot protect it. Folia Biol (praha) 2014;60:275-80.
15. Maia AM, Baby AR, Yasaka WJ, Suenaga E, Kaneko TM, Velasco MV, et al. Validation of HPLC stability –indicating method for Vitamin C in semisolid pharmaceutical/ cosmetic preparations with glutathione and sodium metabisulfite, as antioxidants. Talanta 2007;71:639-43.
16. Chakraborthy A, Ramani P, Sherlin HJ, Premkumar P, Natesan A. Antioxidant and pro-oxidant activity of vitamin C in oral environment. Indian J Dent Res 2014;25:499-504.
17. Brenda Land. Using Vitamin C To Neutralize Chlorine in Water Systems. United staes department of agriculture and forest service, technology and department program, april 2005.
18. Shokouhinejad N, Aligholi M, Amjadi M. Antimicrobial effect of Citrus aurantifolia extract on Enterococcus faecalis within the dentinal tubules in the presence of smear layer. Dental Medicine J. 2011;24(3):148-55